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Factors That Promote the Development of Human Breast Cancer

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Epidemiologic and endocrinologic studies of breast cancer etiology are reviewed in the context of the Moolgavkar two-stage model for mammary carcinogenesis. Promoters are hypothesized to enhance the growth of stem and intermediate cells, and initiators are assumed to cause stem and intermediate cells to give rise to intermediate and tumor cells, respectively. Although all epidemiologic features of breast cancer can be explained in terms of the cellular events supposed by the model, the specific causes of breast cancer are largely unknown. Aberrations in endogenous steroid sex hormones most probably act as promoters, although their exact nature and etiology are unclear. Ionizing radiation is the only known initiator. The two-stage model implies that others exist and that they are responsible for both the international variation in risk of breast cancer, and its familial aggregation. Results of endocrinologic studies suggest either that aberrations in endogenous sex hormones serve as such initiators or are correlated with them, or that familial and international variations in risk are mediated by promoters.

Introduction

Many epidemiologic studies, conducted largely during the past three decades, have resulted in the identification of a number of factors that distinguish women who are at increased risk of breast cancer. A number of investigators have attempted to synthesize information from epidemiologic studies, along with findings from clinical and laboratory investigations, and develop models or hypothesis for the etiology of breast cancer. In spite of these efforts, the specific causes of breast cancer are largely unknown.

The purposes of this paper are to critically summarize the epidemiologic features of breast cancer, describe the more promising etiologic hypotheses and models that have been proposed, and indicate additional studies that might enhance our understanding of the genesis of this disease.

Risk Factors for Breast Cancer

A critical review of the epidemiology of breast cancer was published in 1980 (*1*) and should be consulted for documentation of the risk factors summarized in Tables 1 and 2. Table 1 shows factors that strongly implicate ovarian hormones as etiologic agents. Risk of breast cancer is approximately 100 times greater in women than men. In women, the disease does not occur before menarche. Thereafter the incidence increases rapidly with age until menopause, after which time it increases more slowly with advancing years. Risk of breast cancer is inversely related to age at menarche. This adverse effect of earlier onset of ovarian function appears to diminish with age. Risk also increases with age at menopause, and premenopausal oophorectomy, without exogenous estrogen replacement therapy, reduces the risk of breast cancer, the degree of protection being inversely related to the age at which the ovaries are removed. The adverse consequences of prolonged ovarian function and the protective effect of premenopausal oophorectomy appear to increase with age.

Nulliparous women are at greater risk of breast cancer than parous women, and the risk in women who have had children increases with the age at which their first child is born. Results of some studies show that having additional children may also be weakly protective. The reduction in risk associated with an early first birth may diminish as a woman ages. Lactation does not appear to alter the risk of breast cancer, although in one study women with breast cancer who breast fed from only one breast tended to develop their tumors in the breast that was not used for nursing (*2*).

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Table 1. Risk factors for breast cancer: factors implicating hormones as tumor promoters.

Factor	Influence on risk	Remarks
Female sex	Increases risk	
Age	Increases risk	Effect of age diminishes after menopause
Age at menarche	Inversely related to risk	Influence diminishes with age
Age at menopause	Directly related to risk	Influence increases with age
Oophorectomy	Protection inversely related to age at oophorectomy	Influence increases with age
Nulliparity	Increases risk	
Age at first child	Directly related to risk (early birth protective)	Influence diminishes with age
Parity	Additional children may be weakly protective	
Lactation	Minimal	Unilateral nursing may influence laterality of tumor
Exogenous estrogens	Probably eliminates the protective effect of oophorectomy. Risk in women with intact ovaries uncertain	Results of studies conducted to date are inconsistent

Table 2. Risk factors for breast cancer: probable tumor initiators and factors with unknown mechanisms of action.

Factor	Influence on risk	Remarks
Ionizing radiation	Increases risk	Dose-response, probably linear
Family history of breast cancer	Increases risk	May be multiple explanations
Geography	High risk in "western" countries	Not explainable by known risk factors
Nutritional factors	"Western diet," fats, may increase risk	Effect may be indirect
Weight	May increase risk	Effects mainly postmenopausal disease; not confounded by age at menarche
Height	Questionable increase in risk	Results of studies inconsistent
Body size	Questionable increase in risk	Results of studies inconsistent
Benign breast disease	Increases risk	Lesions with epithelial proliferation and calcification particularly increase risk.

Transvestite males given high doses of estrogens to induce breast development have developed breast cancer, but studies of more moderate doses of estrogens given to women for treatment of menopausal symptoms (*3-7*), or with progestogens in the form of oral contraceptives (*3, 9-11*), have yielded inconsistent results. Estrogen therapy probably eliminates the protective effect of premenopausal oophorectomy (*6, 7, 12*), but results of some studies are not supportive of this statement (*4, 5*).

Table 2 summarizes other factors that have been related to breast cancer. Ionizing radiation is the only known cause of this disease. Although such radiation is undoubtedly responsible for only a minute proportion of all breast cancer, as discussed subsequently, its influence on risk of breast cancer is age-dependent, and this observation has provided insight into possible mechanisms of tumor initiation and promotion.

Risk is approximately doubled in mothers, sisters, and daughters of women with breast cancer, as well as in both their maternal and paternal grandmothers and aunts (*1*). Such familial aggregation could result from either common environmental or genetic factors, and different mechanisms are probably operative in various situations. The comparable risks in maternal and paternal relatives rule out x-linked genetic mechanisms, or transmission of an oncogenic agent via the placenta or breast milk, and the latter is also refuted as a vehicle by the observation that women who were breast fed are not at increased risk.

Evidence for genetic mechanisms are as follows: (a) familially aggregated breast cancers tend to occur in premenopausal women and to be bilateral more frequently than nonfamilial tumors (*13-15*); (b) the risk in sisters and daughters of propositi with breast cancer is enhanced if the propositus has a second-degree relative with breast cancer, is increased even more if she has an affected sister, and is greatest if her mother also had breast cancer (*13, 14*); (c) risk of breast cancer is no greater in dizygotic twins of cases than in other sisters (about doubled), but is increased sixfold in monozygotic twins (*16*); and (d) the lesions occurred on the same side in eight of nine twins that were concordant for breast cancer (*16*).

In spite of these observations, no simple Mendelian mode of inheritance of breast cancer is evident. One exception to this is an observation made in 11 high-risk families in which the pattern of occurrence of breast cancer was compatible with an autosomal

dominant gene chromosomally linked to the glutamate-pyruvate transaminase locus (*17*). This linkage was not found in other high-risk families and probably accounts for only a tiny portion of all breast cancer. It has, nonetheless, provided an important new etiologic lead.

Incidence rates of breast cancer vary among countries by a factor of nearly eight. Rates are highest in Caucasian populations of North America and lowest in parts of Africa, Japan, and among non-Jews in Israel. Migrants from low- to high-risk countries have intermediate rates of breast cancer, and rates gradually approach those of the high-risk country in succeeding generations. In the United States, this "movement" of rates is more rapid in migrants and their decendents from Western Europe than in more culturally distinct groups such as Japanese, Chinese and Mexicans (*1*). These observations clearly show the international differences in rates to be of environmental rather than genetic origin and due to factors associated with a "western" life style. The international differences have been shown not to be due to variations in the prevalence of any of the risk factors that have so far been mentioned. Large increases in rates of breast cancer in succeeding generations of women in Iceland (*18*) and Japan (*19*) have similarly been associated with changes from traditional to modern societies and not to temporal changes in the known risk factors for breast cancer.

Rates of breast cancer have been shown to be correlated with total fat consumption among various countries (*20-22*), temporally and among districts in Japan (*19*) and among five ethnic groups in Hawaii (*23*).

Similar correlations have been observed, although somewhat less consistently, with meat consumption (*19, 21-23*). Case-control studies in Canada (*24*) and among Seventh Day Adventists in the United States (*25*) have also related risk of breast cancer to total fat consumption, and preliminary results from a prospective study in Japan (*19*) show risk to be related to meat consumption. The spouses of Japanese women with breast cancer in Hawaii (and presumably also the cases themselves) were found to have previously consumed more meat and dairy products and less traditional Japanese food than spouses of normal women (*26*). Finally, a high fat diet has been shown to increase mammary tumor production both in rats exposed and not exposed to the mammary carcinogen, 7,12- dimethylbenz(a)anthracene (DMBA) (*27*). Although each observation relating fat and meat consumption to risk of breast cancer can be faulted, in the aggregate they strongly suggest that diets rich in fats and meats, or unknown factors strongly correlated with such diets, increase one's risk of breast cancer.

Studies of the relationship of breast cancer to height, weight, and body size have recently been reviewed (*1*). Findings among studies are inconsistent and difficult to interpret. The most consistent finding (but with several exceptions) is an association of postmenopausal breast cancer with weight. Adult weight is obviously related to nutritional factors and has been inversely correlated with age at menarche and directly related to age at menopause (*28*). The association of weight with breast cancer may thus be indirect.

The risk of breast cancer is roughly doubled in women with previous benign breast lesions. The increase is largely confined to women with fibrocystic lesions characterized by proliferation of ductal epithelial cells (*29, 30*). Risk may be particularly high if there is histologic evidence of calcification of these lesions (*29* and D. L. Page, personal communication).

The Moolgavkar Model

Moolgavkar et al. (*31*) have proposed a two- stage model for the genesis of breast cancer. They hypothesize that malignancies develop from undifferentiated stem cells as a result of two independent events that occur during cell division. A normal stem cell can either die or differentiate, and thus not be at further risk of potentially malignant change, divide into two normal stem cells (each of which are at risk), or divide into one normal cell and one intermediate cell. Intermediate cells can similarly die or differentiate and thus be removed from the pool of cells at risk of a second event, divide into two intermediate cells, or divide into one intermediate and one transformed cell. The transformed cell then grows into a tumor. The exact nature of the hypothesized event is unknown, but may be viewed as a failure of normal cell division in which one of the two daughter cells is not an exact replica of the parent cell. An event may also be viewed as the cumulative effect on a cell of tumor initiators. Tumor promotors, on the other hand, would act by enhancing normal proliferation of stem or intermediate cells.

An elegant mathematical model was developed which included terms representing the net growth rates of stem and intermediate cells and terms for the probabilities of stem and intermediate cells giving rise to intermediate and tumor cells, respectively. By assigning different values to the net cell growth terms, before and after menopause, and different values for the probabilities of events in different human populations, the model accurately simulated the age-incidence curve for breast cancer in six populations with markedly different overall

incidence rates. In biological terms; the international differences in rates would be due to different risks of exposure to tumor initiators; the rapid increase in risk with age between menarche and menopause in all areas would be due to the proliferation of stem and intermediate cells, largely between the ages of 12 and 18; and the decline in the slope of the incidence curves with age after menopause would be due to the decrease in rates of division (or more differentiation or death) of stem or (especially) intermediate cells associated with cessation of ovarian function.

Pregnancy initially causes proliferation, but then marked differentiation of the mammary epithelial cells. The latter would reduce the number of cells at risk of events; and the earlier in life that this happened, the shorter would be the time when large numbers of undifferentiated susceptible cells are present in the breast, and the lower would be the risk of breast cancer. The model accurately simulated the inverse relationship between age at birth of first child and risk of breast cancer.

The other risk factors shown in Table 1 can likewise be explained in terms of the Moolgavkar model as being indicators of hormonal events that promote (or retard) the growth of stem and intermediate cells. Females have more such cells than males and are thus at much greater risk. Early age at menarche as a risk factor is explainable by the fact that large numbers of stem cells are present for a longer period of time in women with an early than late menarche. If the model is correct, risk should be related to the length of time from menarche to birth of first child; and this was observed in a study completed since the model was developed (*32*). Young age at natural menopause or oophorectomy as protective factors are explained by the fact that cessation of ovarian function causes involution of the breast and removal of stem and intermediate cells. To the extent that exogenous estrogens retard this process they would be expected to eliminate the protective effect of a premenopausal oophorectomy or early natural menopause. The possible small protective effect of pregnancies subsequent to the first could be due to slight additional differentiation of the mammary epithelium.

The first three factors in Table 2 most likely represent initiators. Ionizing radiation certainly can cause genetic mutations which could result in events in terms of the model. Knudson (*33*, *34*) has proposed that if the first event in a two-hit model is in a germ cell, then all mammary cells would be intermediate cells and require only one additional event for tumor development. This would explain the younger age at onset of familial cancers and their tendency to be bilateral. The geographic differences in rates of breast cancer were successfully simulated by the model by assuming that they were due to enhancement of the rates of transition, which implies that they are due to differences in levels of initiators in different countries. Weight, height, and body size presumably reflect nutritional status, which is discussed subsequently.

The role of benign breast lesions can be explained in terms of the model by assuming that lesions characterized by epithelial proliferation represent intermediate cells. Noninvasive carcinomas (lobular carcinoma *in situ* and intraductal carcinoma) may similarly be indicative of intermediate cells (*31*).

In summary, the Moolgavkar model nicely relates the epidemiologic features of breast cancer to probable events occurring at the cellular level. Hormonal risk factors are hypothesized to represent promotors which enhance the growth of stem and intermediate cells, and nonhormonal factors are seen likely to represent causes of mutations in individual cells that result in transformations of normal stem cells to intermediate cells and of intermediate cells to fully initiated tumor cells. Intermediate cells are seen to have the ability to proliferate and cause benign or noninvasive tumors but not to invade normal tissues, whereas a fully transformed cell will grow and invade and thus develop into a malignancy.

Although the model provides a logical description of what may happen at the cellular level, it does not tell us what actually causes breast cancer. The specific hormonal aberrations that result in the proliferation of susceptible stem and intermediate cells, and the etiology of these aberrations, are not well understood; and radiation is the only initiator that has been identified. In the next section, our current knowledge of the relationships of breast cancer to specific endogenous hormones will be reviewed, and considered in the context of the proposed model. After that, possible initiators will be similarly considered.

Endogenous Hormones

Estrogens

Estrogens cause proliferation of human breast tissue, so presumably would promote growth of stem and intermediate cells. However, neither breast cancer cases nor individuals at high risk of breast cancer have been consistently shown to have elevated levels of total plasma estrogens or urinary estrogen metabolites.

There are three major endogenous estrogens in humans: estrone (E_1), estradiol (E_2) and estriol (E_3). Until recently, it was thought that estriol was not

estrogenic or carcinogenic in experimental animals and would antagonize the effects of the other two estrogens. Based on this premise, and observations that the ratio $E_3/(E_1 + E_2)$ in urine (the estriol excretion ratio) was low in breast cancer cases, in women with benign breast lesions and in infertile women with anovulatory cycles, and that it was increased during pregnancy, Lemon (*35*) hypothesized that a low urinary estriol excretion ratio (E_3 ratio) facilitated the development of breast cancer. This hypothesis was modified by Cole and MacMahon (*36*) who suggested that a low E_3 ratio between puberty and about age 25 was an important determinant of a woman's lifetime risk of breast cancer. A number of studies were conducted to test this hypothesis by comparing urinary E_3 ratios in members of high and low risk populations. These have previously been reviewed (*1*). In the aggregate they showed, as predicted that young women from high-risk populations have lower ratios than young women from low-risk populations. However, estriol has since been shown to be an active estrogen and a tumor promotor in animal systems (*37*) and to occur in insufficient levels in human plasma to counteract effectively the effects of the other estrogens. Urinary levels of estriol vary due to differences in the final pathways of estrogen metabolism (*38*). The estriol excretion ratio hypothesis must thus be rejected. However, the E_3 ratio must be related to some other factor that is responsible for the international differences in rates of breast cancer. According to the model, this factor acts as an initiator. Therefore, either the model is incorrect, or this factor is nonhormonal, or hormonal aberrations may act as initiators as well as promotors.

In Greece, (*39*), young parous women were also found to have higher urinary E_3 ratios than nulliparous women. This observation suggests that the same basic mechanisms that cause the international differences in rates may also be responsible for the protective effect of an early first child. If so, this too suggests that the international variation in rates is of hormonal origin.

Close relatives of women with breast cancer, who are themselves at increased risk, do not have low urinary E_3 ratios. However, one study (*40*) but not another (*41*) did find elevated plasma levels of $E_1 + E_2$ in relatives of cases, along with elevated plasma prolactin levels. In two studies (*41, 42*), higher levels of $E_1 + E_2$ were found in the urine of relatives of cases than controls. Low levels of androgens in plasma and urine have been observed in sisters of cases (*43*). These findings are difficult to interpret, but do suggest that altered steroid hormone production, metabolism, or excretion, perhaps resulting in more estrogens relative to other hormones, may play a promoting role in some familial breast cancer. This mechanism is different from that proposed by Knudson (*33*), and incorporated into the Moolgavkar model (*31*), by which familial breast cancer was hypothesized to result from the first event occurring in a germ cell and causing an individual to be born with all stem cell precursors already in the intermediate stage.

Progesterone

Progesterone is produced primarily during the luteal phase of the menstrual cycle by the corpus luteum, and causes alveolar cell growth and differentiation in the estrogen primed breast. Sherman and Korenman (*44*) hypothesized that breast cancer was caused by inadequate corpus luteum formation, and hence estrogenic stimulation in the absence of sufficient cyclic progesterone. This hypothesis was subsequently modified by Korenman (*45*), who postulated that menarche and menopause were two "windows" in time when anovulatory cycles are frequent, and individuals are particularly susceptible to environmental carcinogens (initiators, in terms of the Moolgavkar model). Results of some endocrinologic studies are not supportive of this hypothesis: low risk Japanese and high risk British women were found to have similar levels of plasma progesterone and urinary pregnanediol (*46*); daughters of women with breast cancer have been found to have elevated levels of plasma progesterone (*40*) and urinary pregnanediol (*40, 42*); and members of high risk families were not found to have unusual levels of plasma progesterone or urinary pregnanediol (*41*). On the other hand, on the Isle of Guernsey, women at increased risk of breast cancer by virtue of an early menarche, a family history of breast cancer or low urinary androgen excretion, were found, on the basis of low plasma progesterone levels, to have more anovulatory cycles than other women (*47*); and infertile women with anovulatory cycles were found to have higher rates of breast cancer than infertile women with normal cycles (*48*). If the "window" hypothesis is valid, then only endocrinologic studies of women at menarche or menopause would be expected to distinguish high- and low risk women, but this seems not to explain the discrepant findings of studies of progestogens conducted to date.

Androgens

A large prospective study conducted on the Isle of Guernsey has shown that woman who excrete low levels of the androgen metabolites etiocholanolone and androsterone have elevated risks of

subsequent breast cancer (49). Androsterone is androgenic whereas etiocholanolone is not, and low risk Japanese women were found to have a higher ratio of androsterone to etiocholanolone in their urine, indicating that the lower risk women had higher levels of active androgens (50). Sisters of breast cancer cases (43) and 20- to 40-year old women with benign breast lesions, but not older women (51, were found to have lower levels of androgens or their metabolites in their plasma and urine than women at lower risk, although in another study, the urinary levels of etiocholanolone did not distinguish women with benign breast disease from controls (52).

On balance, it seems that low levels of endogenous androgens are associated with an elevated risk of breast cancer. However, on the Isle of Guernsey, the effects on risk of breast cancer of low urinary etiocholanolone level, age at menarche, family history of breast cancer, and age at birth of first child, were independent (on a multiplicative scale) (53). This suggests that low androgen levels are not the mechanism by which the other three factors considered influence the risk of breast cancer.

Prolactin

Prolactin is secreted by the pituitary gland and acts on the estrogenprimed breast to stimulate and maintain lactation. It is thus associated with the functioning of differentiated cells, not the stimulation of stem cell growth, and, like androgens and progesterone, should, if anything, be related to a reduced risk of breast cancer. High levels of prolactin are secreted during pregnancy, and this may be one reason for the reduced risk of breast cancer associated with an early first birth. Most observations, however, suggest that prolactin does not play a role in the genesis of breast cancer. Prolactin is secreted during lactation, but breast feeding seems not to alter one's risk of breast cancer. Neither basal plasma prolactin levels (54-58) nor levels in response to provocative tests (57) have been found to differ in breast cancer cases and controls, and plasma levels in women from high- and low-risk populations were not appreciably different (59). Both reserpine and phenothiazines stimulate prolactin production, and women who have taken these drugs are not at altered risk of breast cancer (1, 60, 61). One study found increased levels of prolactin in daughters of cases (40), but two other investigations failed to confirm this finding (41, 62).

Thyroid

Thyroid hormone probably does not play a direct role in the genesis of breast cancer (1). Studies of women with a variety of thyroid diseases have shown no relationship of breast cancer risk to any type of thyroid condition or treatment (63-65).

In Summary

A simple hypothesis that tumor promotion in the human breast results from either an absolute excess of active estrogens, or an excess relative to other hormones, must be rejected for two reasons: the results of studies of endogenous hormones do not consistently show excess absolute or relative estrogen levels in individuals at increased risk of breast cancer; and studies do not clearly and consistently show an increased risk of breast cancer in women exposed to exogenous estrogens. This is not to imply that endogenous hormones do not act as tumor promotors. They probably do, but their role is complex and not fully understood. Evidence to date suggests that individuals at increased risk for different reasons may have different hormonal aberrations. For example, the aberrations related to international differences in rates appear to differ from those that distinguish women with a family history of breast cancer.

The possibility must also be considered that hormonal aberrations act as initiators. As mentioned in the above section on estrogens, the Moolgavkar model implies that the international variation in rates is due to differences in the prevalence of initiators, and yet such variation has been related to differences in endogenous estrogen (and-androgen) excretion. Alternatively, these observations on hormone excretion may imply that the model is incorrect.

Possible Promotors

Ionizing Radiation

Increased risk of breast cancer has been observed in women who received multiple fluoroscopies of the chest (66-70), women treated with radiation for acute postpartum mastitis (71-73), acne (74), and benign breast diseases (73), women exposed to atomic bombs in Japan (75, 76), and women who worked with paint containing radium (77). The dose-response curve appears to be linear within a wide range of dosages (70, 72, 76, 78), but with some evidence of a leveling off of the curve at high doses. Rad for rad, fractionated and single exposures appear to have about the same effect on risk (78). Breast cancer appears in excess after a latent period of at least 8 years (67). Thereafter, the excess risk persists for up to 30 years after exposure (70, 72). Radiation effects the risk of subsequent breast cancer at all ages to about the same degree (78), al-

though high doses may diminish the latent period somewhat (*72, 73*).

The influence of radiation on risk varies with the age of the women at the time of exposure. Girls exposed prior to breast development are not at increased risk, and those irradiated in early adulthood are at greater risk than women irradiated later in life (*70, 73, 76-78*). In addition, risk of breast cancer was increased to a greater extent in women treated with x-rays for postpartum mastitis following their first child than in women so treated after the birth of other children (*79*).

All of these observations are consistent with the notion that ionizing radiation acts as an initiator in the context of the Moolgavkar model, and that it acts primarily on stem cells to cause the initial event. Thus, radiation has no effect until puberty, when stem cells start to grow and proliferate. Its effect is maximal early in adult life, when stem cells are rapidly proliferating, and diminishes thereafter with age as more stem cells differentiate or die. During the first pregnancy, there is marked proliferation of breast stem cells and therefore a particularly large radiation effect right after this pregnancy. Enhanced differentiation of stem cells then follows the first pregnancy so the effect of radiation after subsequent pregnancies is less. The intermediate cells thus produced by radiation, like any other intermediate cells, are then subject to whatever other initiating factors cause tumor cells to develop from intermediate cells. This mechanism explains why, after an appropriate latent period, risk is enhanced at all subsequent ages, and why cases do not then occur strongly clustered in time like an infectious disease in persons following exposure to a common source. The somewhat shortened latent period in women who received particularly high doses may indicate that, under such circumstances, radiation may also act to induce second events.

The epidemiologic observations on risk of breast cancer in relation to ionizing radiation thus support the Moolgavkar model, and in the context of the model, radiation serves as a prototype for tumor initiators, particularly those acting at stage one. Unfortunately, no other initiators for human breast cancer are known.

Familial Factors

We have seen that the familial aggregation of breast cancer may result from an alteration of endogenous hormones, and if so, that this is at variance with the Moolgavkar and Knudson models which imply that familial breast cancer is due to the first event occurring in a germ cell and causing the individual to have all stem cells in the intermediate stage. On the other hand, there are features of familial breast cancer that are supportive of the model, in particular the early age of onset of familial cases and the tendency for such cases to develop bilateral tumors. It is possible that both mechanisms are operative in different familial situations.

Geographic Factors

Reasons for the marked international variation in rates of breast cancer are unknown. They are undoubtedly environmental and not genetic. In the section on endogenous hormones, it was pointed out that the international differences in rates had been correlated with some measures of endogenous estrogens and androgens, and that these observations suggest that tumor promotors rather than initiators may be responsible for the international differences in rates. On the other hand, the Moolgavkar model implies that the international differences in rates are due to variation in exposure to initiators.

Rates of breast cancer have been correlated with per capita consumption of various animal fats and meats (*20-22*), as well as with gross national product (*20*). These observations could result from the effects of either initiators or promotors, so neither support nor refute the model.

A high fat diet could promote a breast cancer by at least two mechanisms: it could result in obesity, and in obese persons, there is enhanced production of estrone from androstenedione (*80*); or hormone-like substances could be produced by the action of intestinal bacteria on bile salts, both of which may be altered by a high fat diet (*81*). Alternatively, the international difference could be due to unrecognized mammary carcinogens in the environment, the presence of which is correlated with fat or meat consumption. These could be dietary contaminants (*82*), or substances produced in the gut from the action of intestinal bacteria on bile salts or other substances (*83*). Theoretically, they could also be of nondietary origin, although there is currently little evidence for any such substances.

Possible Lines of Further Inquiry

The observations on androgens, estrogens, progesterone, and other hormones are not necessarily in conflict. They may be different indicators of the same (unknown) underlying hormonal aberration. The production, metabolism, and excretion of these hormones, and their resultant concentrations in blood, urine, and breast tissue are certainly interrelated. These interrelationships are complex and not fully understood, and they should be the subject of further study.

More information is also needed on the endocrinologic events associated with such risk factors for breast cancer as a first pregnancy at an early or late age, an early menarche and a late menopause.

Studies to clarify the mechanisms by which individuals with a family history of breast cancer are at increased risk are needed. These should include additional endocrinologic studies to compare various hormones in women with single and multiple first degree relatives with breast cancer, and in women whose relatives have bilateral disease of early onset and unilateral disease of later onset. Other investigations might include epidemiologic studies to ascertain whether the recognized risk factors for breast cancer can explain the increased risk in relatives of cases. Studies of the clonal nature of familial and nonfamilial tumors have been suggested by Moolgavkar (*31*).

Additional studies to attempt to identify causes of the international differences in rates of breast cancer are needed. A search for determinants of a low (or high) urinary estriol excretion ratio might provide a clue. Much more work needs to be, and is being, done on dietary determinants of breast cancer risk. In addition to initiators and promotors, dietary factors that might act to prevent breast cancer should be considered.

It is well known that estrogens increase the amount of calcium in bone. Their influence, and that of other hormones, on the calcification of extraosseous tissues is unknown, and should be studied. Two possible lines of inquiry follow.

The first derives from the observation that women with benign breast lesions characterized by ductal cell hyperplasia with calcium deposits have been shown to be at a particularly high risk of breast cancer (*29*). Perhaps the presence of calcium in these lesions is an indicator of some endogenous hormonal aberration that is also responsible for the development of breast cancer. Hormonal determinants of these mammary calcifications should, therefore, be investigated.

The second suggested line of inquiry derives from a theory for the genesis of breast cancer proposed by Cohen et al. (*84*) in 1978 that has since received little attention. They suggested that calcification of the pineal gland results in decreased production of melatonin, which normally inhibits pituitary gonadotropin and ovarian estrogen production, and that this results in an increase in estrogen levels, which leads to breast cancer. In support of this hypothesis, they cited evidence that the prevalence of calcified pineal glands is correlated with the incidence of breast cancer in various geographical areas. The cause of pineal calcification is unknown. Perhaps this calcification, too, is influenced by estrogens or other steroid hormones. If it is, breast cancer might result from the development of an abnormal positive feedback system in which calcification of the pineal causes a decrease in melatonin, which causes an increase in pituitary gonadotropins, which causes an increase in estrogens (absolute or relative to other steroid hormones), which further increases calcification of the pineal, etc. This mechanism is conjectural, but plausible, and implies that studies of possible hormonal (and other) determinants of pineal calcification would be of value.

Summary and Conclusions

The etiology of breast cancer is complex, multifactorial and poorly understood. The epidemiologic features of the disease are compatible with a two-stage carcinogenic process, with promotors acting to enhance the growth of stem and intermediate cells, and initiators acting to cause stem and intermediate cells to give rise, respectively, to intermediate and tumor cells. Endogenous hormones most probably act as promoters but the specific internal hormonal milieu responsible is unknown. It is not an absolute excess of estrogens, and probably not a simple excess relative to other steroid hormones such as androgens, progestogens, or inactive estrogens. Different aberrations may be operative in different circumstances.

Ionizing radiation probably acts as an initiator, primarily on stem cells. Other initiators undoubtedly exist, but have not been identified. The Moolgavkar model implies that international differences in risk of breast cancer, and familial aggregation of cases, are due to initiators acting on breast stem cells and germ cells, respectively. However, results of endocrinologic studies suggest that hormonal aberrations might be involved. If so, then either the inferences drawn from the model are incorrect and the familial aggregation of cases and the variation in risk among countries are mediated by promotors, rather than initiators, or hormonal aberrations are highly correlated with the presence of initiators, or hormones can act as initiators. Several possible lines of further investigation into the etiology of breast cancer, some to clarify the role of endogneous hormones, have been suggested in this review.

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